Isolation And Characterization Of Terpenoid Compounds Ethanol Extract On Young Coconut Coir (Cocos Nucifera L)

Ni Ketut Sumarni, Asriani Hasanuddin, Siti Nuryanti, Gatot Siswo Hutumo

Abstract: The research on the isolation and characterization of terpenoid compounds from young coconut coir (Cocos Nucifera L.) ethanol extracts has been carried out. The stages of this research include extraction and fractionation, separation and purification by liquid vacuum chromatography and gravity column chromatography. The identification of terpenoids was carried out by thin layer chromatography using a Lieberman-Burchard reagent. Analysis using Fourier Transform - Infra Red spectrophotometry on isolates showed the absorption of the -OH group (3448.72 cm^{-1}), aliphatic CH (2991.59 cm^{-1}), C= O (1762.94 cm^{-1}), C= Cyclic C (1635.64 cm^{-1}), and CO (1101.35 and 1055.06 cm^{-1}).

Index Terms: Ethanol extract, terpenoids, characterization

1. INTRODUCTION

High plants, one of which is coconut (Cocos Nucifera L.), can synthesize various types of secondary metabolites such as phenol compounds and their derivatives, terpenes, and terpenoids, alkaloids, peptides and steroids (Othman, et al., 2019). Igwe & Ugwunnaji (2016) states that the content of secondary metabolites found in the endosperm (inner husk) consists of phenol compounds (0.19 ± 0.04%), flavonoids (0.53 ± 0.08%), alkaloids (0.29 ± 0.01%), tannins (0.18 ± 0.04%) and safonin (0.35 ± 0.06%). Terpenoids are dehydrogenated and oxygenated derivatives of terpenes with the general formula C10H16 and are formed inpenpenes, triterpenes, tetraterpenes, and sesquiterpenes. The terpene compounds that bind to oxygen atoms are called terpenoids (Las, et al., 2003). Terpenes and terpenoids have antimicrobial activity against bacteria, mold, viruses, and protozoa (Irfan, et al., 2014). The secondary metabolite compounds contained in plant mitochondrial cells can be obtained by isolation through the stages of extraction, fractionation, and purification. The extraction step can be carried out maceration using a solvent that is suitable in this case related to the nature of polarity and the type of compound extracted. Altemimi, et al., (2017), states that in dissolving a component of the material, the selection of the appropriate type of solvent can affect the effectiveness and type of compound obtained in the extraction process. Ethanol and methanol solvents are often used in the extraction process, but ethanol solvents are preferred for use in the extraction process. Ethanol can dissolve polar and non-polar compounds, it is volatile, non-toxic, environmentally friendly, economical and selective, as well as methanol solvents have high polarity properties and are able to dissolve most polar chemical compounds capable of inhibiting the damage of compounds phenolic contained in samples of oxidizing enzymes (Widyawati, 2014). Methanol solvents are toxic so that ethanol solvents are preferred for extracting compounds that will be applied as antibacterial. 95% ethanol solvent was chosen in this study because concentration is one of the factors that determine reaction products, the higher the concentration, the interaction between the solvent and dissolved particles is more frequent so it can increase the reaction product.

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2 RESEARCH METHODS

2.1 Tools

The tools used include a set of glassware, Ohaus Pioneer analytical balance, ultraviolet (UV) wavelengths of 254 nm and 366 nm, a set of KKG tools, TLC, KVC, drip pipette, rotary Heidolph Laborota 4000 efficient spectrophotometer, Fourier Transform spectrophotometer -Infra Red (FT-IR) Prestige-21 Shimadzu.

2.2 Material

The materials used include ethanol 95%, hydrochloric acid Pro Analyst (PA), sulfuric acid PA, acetic acid PA, technical solvents: ethyl acetate, methanol, n-hexane, magnesium powder, Meyer and Wagner reagents, Lieberman-Burchard reagents, Merck G-60 silica gel, and TLC plates.

2.3 Sample Preparation

The main sample in this study was young coconut skin waste from Coconut Varieties in Palu, taken from one of the young coconut traders in Jl Muh. Yamin Palu. Samples are cleaned with running water, cut into small sizes, dried and mashed until a 60mesh size is obtained.

2.4 Extraction and Partitioning

Maceration extraction was carried out for 3 x 24 hours of 5,0042 kg of young coconut fiber powder with 95% ethanol solvent. The fibers are vacuum filtered and collected in erlenmeyer and then evaporated with a rotary evaporator and dried with Nitrogen gas bursts. The ethanol extract was partitioned in stages with n-hexane and ethyl acetate as solvent.

2.5 Screening of Secondary Metabolites

Alkaloid Test

The ethanol extract was dropped on two drip plates. One part is used as a control and the second part is dropped with Mayer's reagent and the third part is dropped with Dragendorf

reagent. Alkaloids are positive if the extracts which are dripped by Mayer reagents are white and in the drops that are dragged with yellowish-brown Dragendorf, both are positive containing alkaloids.

Flavonoid Test
Flavonoid test is done by adding concentrated hydrochloric acid and Mg metal to the extract. Positive test if there is a red-orange color.

Saponin Test
Saponin test is carried out by shaking a layer of water in a test tube if formed foam that lasts for about 15 minutes is positive for the saponin test.

Terpenoid and Steroid Test
Test for terpenoids and steroids by means of a sample added anhydrous acetate and concentrated sulfuric acid. Positive tests show that terpenoid compounds form purplish-red and blue and green colors for steroids.

2.6 Isolation and Purification
Isolation and purification of ethanol extract were carried out by chromatographic methods including, Thin Layer Chromatography (TLC), Liquid Vacuum Chromatography (KVC), and Gravity Column Chromatography (KKG). Analysis by TLC used a mixture of ethyl acetate: n-hexane with an appropriate ratio after the compound separation pattern was obtained with a certain Rf value followed by KVC analysis using silica gel G-60 F254 stationary phase and n-hexane: ethyl acetate mobile phase (8: 2) (6: 4) (4: 6) (2: 8); ethyl acetate 100%. The fraction obtained from the results of the KVC was continued with the KKG, previously analyzed by TLC using a mixture of ethyl acetate: n-hexane solvent in the appropriate ratio. The suitable solvent mixture is applied in the GFC repeatedly, the fraction obtained is evaporated and analyzed by TLC until a single compound spot is obtained. Identification of terpenoid compounds used by Lieberman-Burchard spray reagents. Isolates which have the same separation are combined, evaporated to dryness and calculated by weight. The isolates obtained were analyzed using FT-IR spectrophotometer.

TABLE 1
RESULTS OF SCREENING ANALYSIS OF SECONDARY METABOLITES

No	Name of secondary metabolite	Extract	Isolate
1	Alkaloids	+	-
2	Flavonoids	+	-
3	Safonin	+	-
4	Terpenoids	+	+
5	Steroids	-	-

3 RESULT AND DISCUSSION
Samples of young coconut coir powder macerated as much as 5,0024 kg with 95% ethanol obtained 674.06 grams of dry ethanol extract. The results of the screening of secondary metabolites are listed in Table 1.

The results of the analysis of alkaloids with major reagents formed white deposits, this is in accordance with the statement of Marliana et al. (2005). It is estimated that these deposits are potassiumalaloid complexes. The Mayer reagent is a mixture of a solution of mercurium (II) chloride with potassium iodide

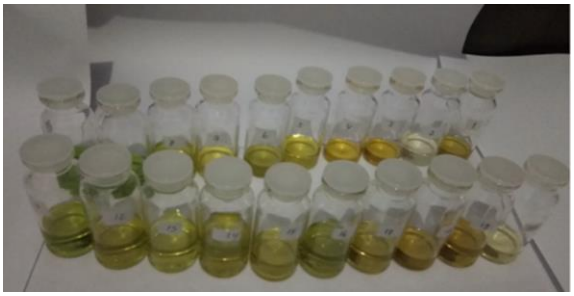

Fig. 1. Liquid chromatography separation fraction results

which reacts to form a red precipitate of mercurium (II) iodide. When potassium iodide is added excess potassium tetraiodomercurat (II) is formed (Altemimi, 2017). Alkaloids contain nitrogen atoms that have lone pairs of electrons so

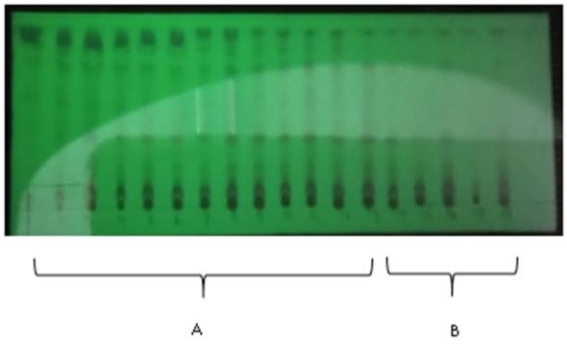

Fig. 2. The pattern of TLC separation results under UV lamps λ245 nm

that they can be used to form coordinate covalent bonds with potassium metal ions to form a precipitating potassium-alkaloid complex (Nurwidayati, 2012). Analysis of flavonoid compounds in the extract occurs through the heating process because it can increase the kinetic energy between particles so that the flavonoid compounds dissolve in aquades, followed by the addition of strong acids, concentrated HCl and

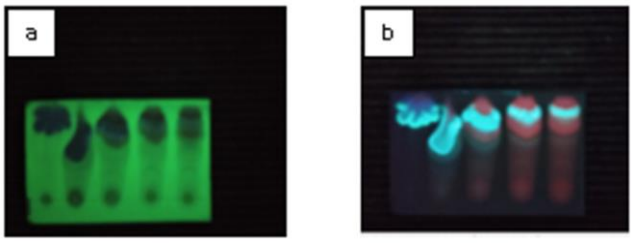

Fig. 3. The TLC separation pattern under the UV lamp: a) λ254 nm; b) λ366 nm

Magnesium powder. Causing a reduction reaction in the benzopiron group to form flavilum salt which is characterized by the formation of orange color (Sembiring et al., 2018). Saponin analysis results are characterized by the formation of foam/froth on the surface of the tube for more than 10 minutes and do not disappear after being shaken with water (Joshi et al., 2013). Saponins are known as surface-active compounds caused by glycosyl which are polar as well as nonpolar steroid or terpenoid groups causing their properties such as surfactants to form stable foam or micelles after adding HCl 2

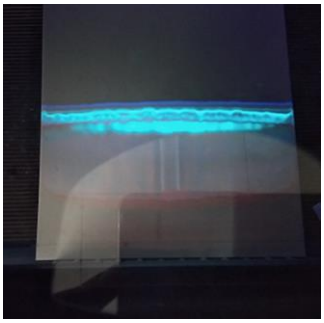

Fig. 4. The preparative TLC separation pattern under UV light λ366 nm

N (Nurwidayati, 2012). The foam formed indicates that there are surface-active compounds that are similar to detergents so that they dissolve easily in water and form foam/froth after being shaken (Joshi et al., 2013). The results of the analysis of terpenoids and steroids with the Liebermann-Bourchad reagent produce a reddish-purple color but react negatively to steroids. The color change indicates a reduction reaction by sulfuric acid in acetic acid anhydride in both compounds. The Lieberman-Burchard reagent is a mixture of concentrated sulfuric acid and glacial acetic acid. The difference in color produced by terpenoids is caused by differences in groups of carbon atoms 4 (C-4) (Kumari et al., 2017). The separation of compounds in ethanol extract was done by thin layer chromatography, liquid vacuum chromatography, and column chromatography. The results of separation by liquid vacuum chromatography (KVC) obtained 18 fractions as shown in Figure 1. After monitoring with TLC, it can be seen the separation of compounds with a pattern as shown in Figure 2. Based on this the same separation pattern are combined so that it can be divided into two fractions, namely fractions A and B. After weighing, obtained fraction A weighing 11.5 grams and proceed with analysis using Gravity Column Chromatography (KKG) with a mixture of ethyl acetate: n-hexane as a mobile phase and silica gel as a stationary phase. The resulting fraction was monitored by TLC and observed under a UV lamp at wavelengths of 254 nm and 366 nm showed a separation pattern as shown in Figures 3a and 3b. The compound which looks fluorescent under UV at 366nm wavelength is then taken and dissolved in ethyl acetate and reanalyzed by preparative TLC, eluted with a mixture of ethyl asset and n-hexane 2:8 ratio. The results show a separation pattern as shown in Figure 4. Preparative TLC results after re-elution with TLC using a mixture of ethyl acetate: n-hexane 2:8 ratio obtained separation patterns as shown in Figure 5. Compounds obtained were relatively pure with a single spot. Identification using a UV lamp at a wavelength of 366 nm is seen in the fading of compounds such as those of terpenoids (Xie et al., 2013). The results of separation with TLC showed that the compounds contained in the isolates were relatively pure. After being dissolved in ethyl acetate and evaporated to dryness, a white needle-shaped solid of 27 mg is obtained. The compounds obtained were analyzed by FT-IR to find out the functional groups contained in the compounds. The spectrum

of FT-IR analysis results is obtained as shown in Figure 6. Based on the spectrum it is seen that there is the absorption of the double bond C=C at wave number 1635.64 cm^{-1}. The IR spectrum also shows the presence of a hydroxy group (O-H) vibration at wave number 3448.72 cm^{-1} and carbonyl vibration (C=O) at wave number 1762.94 cm^{-1}.

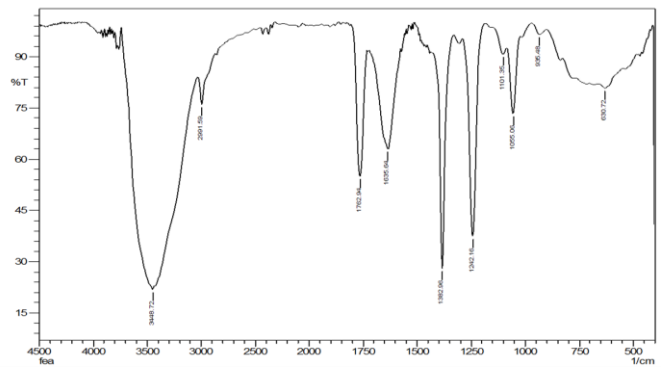

Fig. 6. Spectrum results of FT-IR analysis

This is in accordance with what Maitera et al., (2016) stated that the absorbance at wave numbers 3680 cm^{-1} and 1738 cm^{-1} each indicated the presence of hydroxy groups and carbonyl ester groups. The presence of carbonyl esters in isolate A1.1 was also strengthened by the appearance of C-O vibrations at wave number 1101.35 cm^{-1}. C-H sp^3 vibrations at wave number 2991.59 cm-1 indicate the existence of aliphatic C-H group stretching vibrations that indicate the presence of methyl ($-CH_3$) and methylene ($-CH_2$) groups. This data is strengthened by the presence of C-H buckling vibrations at wave numbers 1382.96 cm^{-1} and 1242.16 cm^{-1} which indicate the presence of dimethyl groups that are specific to terpenoids (Mohandas et al., 2018).

4 CONCLUSION

Based on the results obtained, it can be concluded that the secondary metabolites that were isolated from the ethyl acetate fraction from young coconut coir (Cocos Nucifera L) are isolates containing white crystals containing needles

Fig. 5. The TLC pattern preparation under UV lamps λ366 nm

weighing 27 mg. The results of the collection of compositions based on FT-IR data showed the presence of the -OH functional group (3448.72 cm^{-1}), C-O alcohol (1101.35 and 1055.06 cm^{-1}), CH aliphatic alkane (2991.59 cm^{-1}), C=C (1635.64 cm^{-1}) alkenes and C=O ketones (1762.94 cm^{-1}). Based on the expected interpretation data, the isolates are terpenoids.

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